LOCAL regression applied to a citrus multispecies library to assess chemical quality parameters using Near Infrared Spectroscopy

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ABSTRACT

The non-destructive on-tree measurement of the chemical quality attributes of fruits belonging to the Citrus genus using rapid spectral sensors is of vital interest to citrus growers, allowing them to carry out a selective harvest of any species of citrus fruit. With this objective, the viability of using of a handheld portable near infrared spectroscopy (NIRS) instrument to predict soluble solid content (SSC), pH, titratable acidity (TA), maturity index and BrimA, in order to measure the optimum harvest time in a group made up of 608 samples belonging to the Citrus genus (378 oranges and 230 mandarins) was evaluated. For each of the parameters analysed, both non-linear regression (LOCAL algorithm) and linear regression (Modified Partial Least Squares, MPLS) strategies were designed and compared. The use of the LOCAL algorithm in the sample group of oranges and mandarins for all the parameters analysed allowed to obtain more robust models than those obtained with MPLS regression, and it could also be extended more easily when routinely applied. The results confirm that NIRS technology combined with non-linear regression strategies such as the LOCAL algorithm can indeed respond to the needs of the citrus growers and help them to set the optimum harvest time, in this case of oranges and mandarins, by predicting the chemical quality parameters *in situ*.

Keywords: NIR spectroscopy; Citrus genus; *In situ* analysis; Chemical quality; LOCAL algorithm; Optimum harvest time.

1. Introduction

Since oranges and mandarins are non-climacteric fruits, the harvest should be timed for when the fruit has reached its commercial maturity [1].

For both these citrus species, the harvest indices generally used are based on maturity index values (ratio of SSC to TA), BrimA (an abbreviation for Brix minus Acids) and a minimum yellow-orange colour of the peel [2–5].

On-tree intact measurement of these harvest indices for all the fruits is particularly critical for these non-climateric fruits due to the fact that the physiological maturation process has finished at harvest, and since flavour perception of these fruits is closely linked to these quality attributes (SSC and TA) [4, 5]. Since consumer acceptance of the fruits is based on flavour and sweetness, measuring these values of the fruits on the tree would allow them to be harvested selectively and then sold according to their quality [6].

Therefore, due to the need to test and measure the chemical quality parameters of individual Citrus fruits, the Citrus sector requires the introduction of non-destructive, fast, versatile, environmentally-friendly and cost-effective technologies such as NIR Spectroscopy, which allows to measure the quality of the fruit directly on the tree during the maturation process, regardless of the species analysed.

Most applications which use NIR spectroscopy to measure quality chemical parameters (SSC, pH, TA, maturity index and vitamin C) in fruits of the Citrus genus refer to studies carried out with laboratory equipment for a single species using linear regression techniques, such as Partial Least Squares (PLS), Multiple Linear Regression (MLR) and Principal Component Regression (PCR) [7–10].

However, very few works have focused on taking readings directly on the tree to establish the optimum harvest time [11-13]. Sánchez et al. [11, 12] used portable equipment based on micro-electro-mechanical system (MEMS) technology, with a 1600-2400 nm spectral range to measure quality parameters (SSC, TA, pH, maturity index) in mandarins and oranges separately. Similarly, Cavaco et al. [13] measured the on-tree quality of oranges through internal quality parameters (SSC, pH, TA and maturity index), using portable VIS/NIR equipment with a charge-coupled device array detector and a reduced range of measurement (680-1100 nm).

In addition to *in situ* measurements, it would be highly advantageous in practical and commercial applications to be able to use universal equations for different citrus species, to measure physical-chemical quality, thus permitting the staggered collection of the fruits depending on when they reach their full maturity. Despite this need for universally-applicable equations, there are few published works which refer to developing NIRS models for multi-product groups in plants [14-17], and the work published by Torres et al. [17] is the only one dealing with analysing citrus species intact on the tree as a way of measuring the morphological and physical quality of the fruits.

In the case of heterogeneous spectral libraries (multispecies libraries), the application of non-linear regression methods based on local calibrations allow a better management of the population available, since the characteristics of the samples selected by the algorithm to be used for calibration are specific in each case and for each of the samples to be predicted, thus making it easier for producers to develop models [17-20].

A number of works, in products which are not fruits, have confirmed that the use of non-linear regression techniques with multispecies libraries allows to obtain models with a higher predictive capacity and, most importantly, facilitates the routine management of prediction models and especially their recalibration, since it is simply a case of expanding the calibration database, rather than having to recalculate the models as in classic global strategies. Thus, Godin et al. [21] compared their results obtained by applying non-linear and linear regression methods (LOCAL *versus* MPLS algorithms) to predict neutral and acid detergent fibre residues, acid detergent lignin and mineral compound content in a set composed of different fibrous plants. They concluded that the reliability of non-linear models is greater, since they fit in better with the non-homogeneity associated with a multispecies database.

Similarly, in fruits, the potential of local regression techniques for increasing the robustness of prediction models has been demonstrated by different authors, although these models have been developed for individual species [12, 22-24].

In the particular case of citrus species (oranges and mandarins), Torres et al. [17] applied the LOCAL algorithm in a previous work to measure morphological parameters (weight, equatorial and axial diameter), colour (L*, a*, b*, C* and h*) and physical parameters (firmness, pericarp thickness and juice mass). When this non-linear regression algorithm was applied instead of the MPLS regression, the predictive capacity of the models increased for all parameters and the prediction error decreased.

The aim of this study was to develop predictive models based on non-linear regression strategies (LOCAL algorithm), in order to measure the main chemical quality parameters which indicate the optimum harvest time and allow to carry out selective harvesting in fruits of the Citrus genus, regardless of the species, growing-season and crop practices, using NIRS technology together with a handheld portable MEMS-NIR spectrophotometer.

2. Material and Methods

2.1. Fruit Samples

The initial sample set comprised 608 samples belonging to the genus Citrus – 378 oranges (*Citrus sinensis* L. cv. 'Powell Summer Navel') and 230 mandarins (*Citrus reticulata* Blanco cv. 'Clemevilla') – grown in a commercial plantation in La Campana (Seville, Spain), under four different irrigation regimes.

In the case of orange, each experimental plot comprised three rows of four trees, with four repetitions for each irrigation regime; oranges were monitored on the two central trees in each plot. Thus, ripening was monitored on eight trees for each of the four irrigation regimes, giving a total of 32 trees. A total of six oranges were labeled on each of the 32 trees: one for each of the four possible orientations (north, south, east, and west) and one for each of two heights on the tree (1.25 and 1.75 m), thus giving a total of 384 oranges. However, in the course of the study, six ripe oranges dropped off the tree and were thus excluded. The final sample set thus comprised 378 oranges.

For mandarins, each experimental plot comprised three rows of four trees, with four repetitions for each irrigation regime; oranges were monitored on the two central trees in each plot. Thus, ripening was monitored on eight trees for each of the four irrigation regimes, giving a total of 32 trees. A total of eight mandarins were labeled on each of the 32 trees: one for each of the four possible orientations (north, south, east, and west) and one for each of two heights on the tree (1.25 and 1.75 m), thus giving a total of 256 oranges. However, in the course of the study, twenty-six ripe mandarins dropped off the tree and were thus excluded. The final sample set thus comprised 230 oranges.

On arrival at the laboratory, the harvested oranges and mandarins were kept in refrigerated storage at 5ºC and 90% RH until the following day, when laboratory testing was performed. Prior to each test, the samples were allowed to reach room temperature of 20ºC, suitable for conducting the analysis.

2.2. Reference Data

The chemical parameters (SSC, TA and pH) of the oranges and mandarins were measured in the same way as Sánchez et al. [11]. The maturity index was also calculated as an SSC/TA ratio and the BrimA index was calculated using the equation described by Jordan el al. [4]:

$$BrimA = SSC - k(TA),$$

where k is a constant that reflects the tongue's higher sensitivity to TA compared to SSC. The value of the constant k was 4, which was suggested for oranges by Obenland et al. [5] in order to avoid the generation of negative BrimA values.

2.3. NIR Analysis

NIR analysis of both fruits were performed in reflectance mode (log 1/R) using a handheld MEMS spectrophotometer Phazir 2400 (Polychromix, INC., Wilmington, MA, USA) that incorporates all the essential components to deliver on-tree applications. This instrument scans at 8 nm non-constant intervals in the spectral range 1600-2400 nm. Four spectral measurements were made for each fruit (orange and mandarin) in the equatorial zone whilst on the tree, taking orientation (north, south, east and west) into account. The four spectra were averaged to provide a mean spectrum for each sample.

2.4. Definition of Calibration and Validation Sets

Principal component analysis (PCA) was performed on each individual data set (378 oranges and 230 mandarins) in order to structure and compress the data matrix. After PCA, the centre of the spectral population was fixed in order to detect outlier samples. The Mahalanobis distance (GH) was calculated between each sample and the centre of the population. Samples with a GH value greater than 4 were considered outliers [25]. As signal spectral pre-treatments, the standard normal variate (SNV) plus detrending (DT) procedures [26] were used to remove the multiplicative interferences of scatter, and the Norris first derivative mathematical treatment was performed (1,5,5,1), where the first digit is the order of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing and the fourth is the second smoothing [27].

After removing the outliers (in this case, 3 oranges and 1 mandarin), each of the resulting sets, consisting of 375 oranges and 229 mandarins, was divided into two: a calibration set containing about 75% of the samples and a validation set containing the remaining 25%. These samples were selected following the method outlined by Shenk and Westerhaus [28] using the CENTER algorithm included in the WinISI II software package version 1.50 to calculate the distance to the centre of the population based on the Global Mahalanobis distance (GH), with three out of every four samples selected to be part of the calibration set [29]. Additionally, the calibration and validation sets of oranges and mandarins were merged to make new calibration and validation sets of citric fruits with the two species tested together. The differences in the number of samples available for the different parameters analysed in both the calibration and validation groups were due to the fact that, in some of them, the pH and TA measurements or the parameters derived from titratable acidity (maturity index and Brim A) could not be recorded since the fruits had a very low juice content.

2.5. Construction of Prediction Models using the LOCAL Algorithm. Comparison with Models Obtained Using Linear Regression Strategy

The LOCAL algorithm was performed for each dataset (oranges, mandarins and oranges and mandarins). LOCAL operates by searching for, and selecting, samples in large databases containing spectra similar to the sample being analysed. The selected samples are then used to compute a specific calibration equation, based on PLS regression, to predict the constituents of an unknown sample [18].

Selection of the calibration samples is controlled by the value of the coefficient of correlation between the spectrum of the unknown sample and those comprising the spectral database [18]; the samples with the highest correlation are selected. A minimum correlation cut-off is available to ensure that the selected samples are highly correlated [30].

Different parameters must be evaluated in order to optimize the LOCAL algorithm. In this work, an optimization design was set up by varying the number of calibration samples (k) from 80 to 140 in steps of 20, and the number of factors (l) from 14 to 16 in steps of 1. This gave a factorial design of 4 x 3 or 12 runs. Finally, it was established that the first four PLS factors should be removed.

Furthermore, for each parameter analysed, the different mathematical signal pre-treatments were evaluated. For scatter correction, the SNV and DT methods were tested

[26]. Additionally, four derivative mathematical treatments were tested in the development of NIR calibrations: 1,5,5,1; 2,5,5,1; 1,10,5,1; 2,10,5,1 [27].

The effect of the different settings on the performance of the LOCAL algorithm was evaluated by comparing the standard error of prediction (SEP) obtained for each set, the coefficient of regression for external validation (r^2_p) and the RPD_p (ratio of the standard deviation (SD) of the reference data for validation to the SEP).

In addition, in order to compare the results obtained with the LOCAL algorithm, global models using linear regression were developed.

To achieve this, MPLS regression was used to obtain equations for each data set and for each parameter analysed [25]. During the development of the MPLS equation, the same signal pre-treatments used with LOCAL algorithm were used (SNV + DT, and the four derivative mathematical treatments). The best predictive models obtained for the calibration sets, selected by statistical criteria (the standard error of cross validation (SECV) and the coefficient of determination for cross validation (r^2_{cv}), were subjected to evaluation using the validation sets, which consisted of samples not involved in the calibration procedure.

The SEP values of the predictive models for the parameters tested obtained using the LOCAL and MPLS regression algorithms were statistically compared using Fisher's F test [31]. The values for F were calculated as:

$$F = \frac{SEP_2^2}{SEP_1^2}$$

where SEP_1 and SEP_2 are the standard error of prediction of two different models and $SEP_1 < SEP_2$. F is compared to $F_{critical}$ (1- P, n_1-1, n_2-1) as read from the table, with P = 0.05 and n_1 the number of times the measurement is repeated with method 1; n_2 is the number of times the measurement is repeated with method 2. If F is higher than $F_{critical}$, the two SEP values are significantly different.

3. Results and Discussion

3.1. Population Distribution of Chemical Quality Parameters

Perez-Marín et al. [20] showed the importance of the population distribution used in calibration to obtain robust models. For multispecies or multiproduct groups, using local rather than global calibrations has particular advantages in those parameters where different populations are observed for each species [17].

The distribution of the chemical quality parameters tested for oranges, mandarins, and oranges and mandarins, is shown in Fig. 1, together with their mean and standard deviation. Since the maturity index and BrimA parameters are obtained from the SSC and TA content, in the discussion the distributions shown for the latter, together with their pH values are focused on.

For SSC, the set composed of oranges shows a non-normal distribution, more similar to a bimodal distribution, with a valley around 12% and a maximum around 10.5%, while mandarins show a normal distribution, with a maximum around 12.5%. It could be said that mandarins (ranging from 9.95 to 15.65) were sweeter than oranges (ranging from 6.80 to 15.30). If the groups of oranges and mandarins are joined, a new group is formed (oranges and mandarins) with a distribution close to normal, with a range between 6.80 and 15.6% and a maximum around 12.5%.

In the case of pH and similar to SSC, the mandarins group shows a normal distribution with a range from 2.08 to 4. The oranges group also has a normal distribution, with a range of 3.01-4.15. Since there are more oranges than mandarins, when the two groups are joined, the average value (3.53) is closer to that of the oranges group (3.69), and its deviation (0.30) is higher than that of both groups (0.20 in both cases) and losing the normal distribution.

Taking the groups of oranges and mandarins individually, they show a normal distribution for titratable acidity, with maximum values of 0.60 and 1.10% of citric acid for oranges and mandarins, respectively. For both groups together, there is a positive asymmetric distribution, with a clear maximum value around 0.60% for citric acid and a standard deviation of 0.34% citric acid.

3.2. Descriptive Data for NIR Calibration and Validation Sets

As it was explained in the Material and Methods section, the CENTER algorithm was applied to the individual spectral databases in order to structure the populations according to GH. A total of 3 oranges and 1 mandarin presented values of GH greater than 4, and these were therefore considered outliers. A detailed analysis of the chemical characteristics of these samples could determine that these samples have different characteristics from the rest; the three oranges considered as outliers had low values of SSC (7, 7.35 and 9.11%, respectively), being cases of samples collected before complete maturation, whereas the mandarin sample showed a high value of SSC (15.45%), being a sample collected in an over-ripe state.

Once the outliers have been removed, the remaining samples were used to create the calibration and validation sets. The statistics obtained (number of samples, range, mean, standard deviation and coefficient of variation) for each of the parameters analysed in the calibration and validation sets for oranges, mandarins and the set composed by oranges and mandarins are shown in Table 1. For each parameter, the ranges for the validation set lay within the range for the calibration set; it could be affirmed that the validation set comprised representative samples of the whole variance. Furthermore, both sets of the same group of samples displayed similar values for mean, SD and CV.

For both the calibration and validation groups, the group that has the greatest variability is the one consisting of oranges and mandarins for the TA, pH and maturity index and in the case of SSC and BrimA, the variability of the oranges set is practically identical to that of the oranges and mandarins set.

3.3. Optimization of Settings for the Development of Predictive Models using the LOCAL Algorithm

The SEP values obtained for the best mathematical treatments for the set composed of oranges and mandarins using the LOCAL algorithm, for each one of the combinations of the number of samples (k) and the number of PLS factors, are shown in Fig. 2. It must be highlighted that LOCAL was tuned (i.e. the pre-treatments, numbers of factors and calibration set size) on the validation set. This could give LOCAL a slight advantage over PLS; in this case PLS was tuned by the cross-validation.

As regards the SSC parameter, it can be seen in Fig. 2 that, when 16 PLS factors are used, the SEP value increases as the number of samples increases, while for 14 and 15 factors, there is a slight decrease in SEP when the number of samples reaches 120; the lowest SEP value is obtained when 80 samples and 16 PLS factors are used. This shows that when there is a group with a uniform distribution (Fig. 1), the LOCAL algorithm used fewer samples (80 samples) for predicting the external validation set than the global regression techniques (456 samples), since only those samples whose spectra were considered representative of the sample of the calibration set to be predicted were used. It should also stress the importance of having a large sample group with a wide variability in order to obtain robust prediction models, since having a wide, varied spectral library available, thanks to the samples selected for development from the specific models carried out by the LOCAL algorithm, allows to obtain better prediction results [23].

As it is shown in Fig. 2, the pH does not follow a fixed trend in terms of the evolution of SEP values obtained and the number of samples used to develop the models, and the lowest SEP value (0.15) is obtained when 100 samples and 16 PLS terms were used. For titratable acidity, the lowest SEP value (0.14% citric acid) is obtained when 80 samples and 14 PLS factors are used. In general, it could be said for both parameters that the more samples used, the higher the value of SEP obtained.

For maturity index and BrimA, the SEP values decrease as the number of samples used increases, and the lowest SEP values for both parameters are obtained initially when 140 samples are used (Fig. 2). The need for a greater number of samples shows that these modelling parameters are more complex, since they are derived from the relationship between simpler ones, such as SSC and TA. In addition, since in this case it was not clear if the minimum SEP value had been obtained with the number of samples tested (up to 140), it was decided to extend the number of samples used to evaluate this optimization parameter of the model (number of samples, k) to 200. For the maturity index, the minimum SEP value was obtained with 160 samples and 16 PLS factors, while for the BrimA parameter, the lowest SEP value was obtained with 140 samples and 14 PLS factors. It can therefore be confidently asserted that the lowest SEP values for maturity index and BrimA are 2.98 and 0.84, respectively (Fig. 2 and Table 2).

3.4. Validation Statistics for Predicting Chemical Quality Parameters in Citrus Fruits using the LOCAL and MPLS Algorithms

The validation statistics used to predict the chemical quality parameters in oranges, mandarins, and oranges and mandarins using LOCAL and MPLS regression algorithms are shown in Table 2. This table shows SEP, r^2_p, RPD_p and the settings (LOCAL algorithm) used for the best mathematical treatment for both regression strategies.

The set including all the samples (oranges and mandarins) obtained a good predictive capacity for all the parameters tested using the LOCAL algorithm, displaying values of r^2_p between 0.72 and 0.84 [32]. In general, the values of r^2_p obtained with the non-linear regression algorithm for the set composed of both species are greater than the values obtained for the individual sets, except for the set of oranges in the case of SSC and BrimA, and the set of mandarins for pH and maturity index, whose r^2_p values are slightly higher.

Furthermore, the validation statistics used to predict the chemical quality parameters show that models obtained using the LOCAL algorithm improved the predictive capacity (higher values of r^2_p) and the accuracy (lower values of SEP) with respect to MPLS regression for all the parameters, except for titratable acidity and maturity index in the set composed of oranges, whose predictive ability (r^2_p values) using LOCAL algorithm fell by 4% and 3%, respectively. For the other models developed, the improvement obtained with the LOCAL algorithm was 7–17% for r^2_p, with the mandarins group the highest for the SSC parameter and the oranges group for pH, with 46% and 67%, respectively; in the same way, the decrease in SEP values when applying the non-linear regression algorithm ranged from 4 to 18%, except in the case of pH for the mandarins group and titratable acidity in oranges, where there was no difference in terms of the errors obtained with the algorithms tested.

On the other hand, comparisons using Fisher's F test of the SEP values in the models obtained for the different parameters analysed, using different regression strategies (LOCAL and MPLS algorithms) for the groups of oranges, mandarins, and oranges and mandarins, pointed to the existence of significant differences ($P < 0.05$) for the SSC parameters in the oranges group, and for titratable acidity and maturity index both in the mandarins and the oranges and mandarins groups. For the other remaining parameters, the differences in SEP values were not significant ($P > 0.05$) (Table 2).

As regards the SSC and BrimA parameters, although there were no significant differences between the SEP values when applying the LOCAL algorithm or MPLS in the group of oranges and mandarins, Fig. 1 clearly shows that the range available for the oranges group covers that of the mandarins and makes no distinction between the populations. For this reason, there are no important benefits is applying local regressions, except for the advantages of a routine handling of the spectral databases and the possibility of updating the models more easily if LOCAL is used.

In terms of r^2_p and considering the LOCAL algorithm, the SSC models obtained a good predictive capacity for oranges ($r^2_p = 0.81$) and for the set composed of oranges and mandarins ($r^2_p = 0.78$), whereas in the case of mandarins, the model constructed could only distinguish between low, medium and high values ($r^2_p = 0.57$) [32]. However, according to Nicolaï et al., [33] the RPD_p values obtained for the models developed for oranges (RPDp = 2.23) and for the oranges and mandarins group (RPD_p = 2.09) indicate that coarse quantitative predictions are possible for this parameter (RPD_p = 2–2.5), while the model obtained for mandarins (RPD_p = 1.51) can discriminate low from high values (RPD_p = 1.50–2.00). This reduced capacity obtained for the mandarins group can be attributed to its lower variability, according to the CV value given (Table 1). As shown in Table 2, the predictive capacity obtained for the oranges and mandarins group is very similar to that of the oranges group, and there are no significant differences ($P > 0.05$) between their SEP values, which stresses the effectiveness of the LOCAL algorithm to measure SSC in two species simultaneously, using the same equipment and prediction model.

The only study found in the bibliography which measures SSC in a multispecies group of the Citrus genus was the work by Clark [15], who analyzed a group made up of samples of grapefruit, interspecific hybrids (including kumquats, orangequats and citranges), lemon-lime, mandarins and oranges, using FT-NIR (Bruker Alpha spectrometer) equipment and applying PLS regression. This author, however, analyzed samples of the juice, which is much more homogeneous than the whole fruit.

For the prediction of pH and titratable acidity, the results obtained for the oranges and mandarins group show a good predictive capacity for both parameters (r^2_p = 0.72 and RPD_p = 1.93 for pH and r^2_p = 0.84 and RPD_p = 2.43 for TA) using the LOCAL algorithm [32], while for RPD_p, the models developed for these parameters allow to distinguish between high and low pH values and to make a coarse prediction for TA [33].

With the LOCAL algorithm, the predictive capacity improves considerably both for pH and for titratable acidity in the oranges and mandarins group compared with the oranges group. When both species are taken together, r^2_p increases by 188% and 87%, for pH and TA respectively, compared with the oranges group, which could be due to the increase in range which occurs when mandarins are added to the oranges group.

In the same way, there is also a 10% improvement in the accuracy of the model for titratable acidity compared with the mandarins group (r^2_p= 0.76), while for pH, with both groups combined, there is a significant increase in the SEP value (around 36%) compared with the mandarins group, which may be caused by the fact that, when both species are taken together, the mean value is higher than that of the latter group.

As regards the maturity index and BrimA parameters for the oranges and mandarins group, both parameters have r^2_p values of 0.70 - 0.90, thus showing a good predictive capacity [32]. In terms of SEP, when LOCAL is applied to all the samples, the error decreases relative to the oranges group, while there is a significant increase in the error ($P < 0.05$) compared with the mandarins group: 163% and 20% for maturity index and BrimA, respectively. However, these SEP values refer to mean values of uncertainty, which means that they vary depending on the mean of the calibration group used to produce each individual model, although individual uncertainty values can vary, being in some cases higher and in others lower [34]. Nevertheless, this lack of precision is to a large extent compensated for by the opportunity of having a model which includes different species, which is of great interest to the citrus fruit industry. In the same way, although maturity index and BrimA are two parameters related to the perception of sweetness or tartness in the fruit, different authors have defined the latter as more useful [4,5], and it obtained a slightly higher predictive capacity than that of the maturity index (RPD_p = 2.15 for BrimA *versus* RPD_p = 2.08 for maturity index) when LOCAL algorithm is applied.

In general, it is important to stress the usefulness of the LOCAL regression algorithm compared with the linear regression algorithm MPLS to predict chemical quality parameters in the oranges and mandarins group. In particular, as mentioned by other authors [12, 23, 35], the most important factor is the increased robustness attained when applying the LOCAL algorithm to measure quality parameters in fruits, which is notable in this work in the case of pH and titratable acidity parameters, which are both of great interest for the industry and the consumers of these products.

There are no references in the bibliography to authors applying LOCAL regression models in order to measure chemical parameters in groups made up of several species of citrus fruit. However, a number of authors have demonstrated the potential of local regression techniques to measure chemical parameters in oranges [12], grapes [22], nectarines [23], and apples [24], all of which show increased precision and accuracy when non-linear regression techniques are used, as opposed to linear ones.

3.5. Effective Wavelengths for the parameter BrimA

Given the value of the BrimA parameter to the citrus industry [5], it was considered important to study the wavelengths that influence its measurement.

To do this, the loading plot corresponding to the best model obtained using MPLS regression to predict BrimA in a set composed of oranges and mandarins using the Phazir 2400 is shown in Fig. 3. This figure shows the areas of the spectral range where covariance has influenced the computing of the MPLS model to a greater or lesser degree, and the direction (positive or negative). A representation of the latent variables (LV5 to LV8) used in constructing the calibration equation shows that the areas of the spectrum exerting higher weight on model were 1730, 1830, 1900 and 2350 nm, related to the absorption of glucides and water [36].

4. Conclusions

These results confirm that NIR spectroscopy could be an advantageous technique to predict chemical parameters in a set composed by two species belonging to the Citrus genus using the LOCAL regression algorithm in order to establish the quality and maturity indexes of the citrus fruits on-tree. Using the LOCAL algorithm not only represents an improvement in the predictive capacity of the models obtained, but also allows to use multispecies spectral libraries. This is extremely important for the citrus fruit sector, as the libraries can easily be extended to include other citrus species, thus allowing us to obtain universal models. In addition, the results confirm the advantages of using portable equipment which allows to analyse the fruit in the field, in order to harvest the fruits selectively at the optimum time and to obtain a product of the highest quality which is intended both for fresh consumption and for the processing industry.

From a practical point of view, this could be extremely useful for citrus growers, since it permits them to measure maturity indices such as BrimA quickly and without damaging the fruit, which is essential for setting the optimum harvest time and producing fruit which is acceptable to the consumers.

Acknowledgements

The authors would like to thank Prof. Elías Fereres and Dr. Victoria González-Dugo. We are also grateful to Mrs. Mª Carmen Fernández of the Animal Production Department for her technical assistance. This work was funded by the Spanish Ministry of Science and Innovation for the projects CONSOLIDER CSD2006-0067 and AGL2012-40053-C03-01, with participation of funds from FEDER (European Union) and from the Andalusian Regional Government under the Research Excellence Program, project no. P09-AGR-5129 'MEMS and NIRS-image sensors for the in situ non-destructive analysis of food and feed'. Furthermore, the authors wish to express their gratitude to the Spanish Ministry of Education, Culture and Sports for the support offered to Irina Torres in the form of the Training Programme for Academic Staff (FPU).

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Table 1

Statistics for each set and parameter.

Parameter	Samples	Set	Number of samples	Range	Mean	SD	CV (%)
Soluble solid content (%)	Oranges	Calibration	283	6.80-15.30	10.73	1.91	17.80
		Validation	92	7.50-14.35	10.68	1.78	16.67
	Mandarins	Calibration	173	9.95-15.65	12.51	1.19	9.51
		Validation	56	9.95-15.00	12.58	1.07	8.51
	Oranges + mandarins	Calibration	456	6.80-15.65	11.41	1.88	16.48
		Validation	148	7.50-15.00	11.40	1.80	15.79
pH	Oranges	Calibration	283	3.01-4.15	3.69	0.21	5.69
		Validation	92	3.28-4.03	3.70	0.18	4.86
	Mandarins	Calibration	166	2.08-3.80	3.25	0.20	6.15
		Validation	55	2.86-3.69	3.26	0.21	6.44
	Oranges + mandarins	Calibration	449	2.08-4.15	3.52	0.30	8.52
		Validation	147	2.86-4.03	3.54	0.29	8.19
Titratable acidity (% citric acid)	Oranges	Calibration	282	0.36-1.21	0.62	0.14	22.58
		Validation	92	0.37-1.02	0.62	0.15	24.19
	Mandarins	Calibration	155	0.68-2.15	1.21	0.28	23.14
		Validation	50	0.79-1.77	1.89	0.27	14.29
	Oranges + mandarins	Calibration	437	0.36-2.15	0.83	0.34	40.96
		Validation	142	0.37-1.77	0.82	0.34	41.96
Maturity index (SSC/TA)	Oranges	Calibration	282	8.24-40.03	18.14	5.42	29.88
		Validation	92	8.55-35.79	18.55	6.02	32.45
	Mandarins	Calibration	155	5.41-17.27	10.86	2.32	21.36
		Validation	50	6.68-15.68	11.00	2.42	22.00
	Oranges + mandarins	Calibration	437	5.41-40.03	15.56	5.74	36.89
		Validation	142	6.68-35.79	15.59	6.21	39.83
BrimA index (%)	Oranges	Calibration	282	4.29-13.31	8.26	1.93	23.37
		Validation	92	4.63-12.22	8.22	1.98	24.09
	Mandarins	Calibration	155	2.93-10.33	7.70	1.42	18.44
		Validation	50	4.63-10.28	7.75	1.40	18.06
	Oranges + mandarins	Calibration	437	2.93-13.31	8.06	1.73	21.46
		Validation	142	4.63-12.22	8.05	1.81	22.48

Table 2

Validation statistics for predicting chemical quality parameters in Citrus fruits using non-linear (LOCAL) and linear (MPLS) regression algorithms and standard errors of laboratory (SEL)

Parameter	Set	LOCAL				GLOBAL			F	$F_{critical}$	SEL
		Settings	SEP	r^2_p	RPD_p	SEP	r^2_p	RPD_p			
Soluble solid content (%)	Oranges	100, 16, 4	0.80	0.81	2.23	0.97	0.75	1.84	1.47	1.40*	0.11
	Mandarins	140, 16, 4	0.71	0.57	1.51	0.84	0.39	1.27	1.40	1.43	0.07
	Oranges + mandarins	80, 14, 4	0.86	0.78	2.09	0.95	0.72	1.89	1.22	1.40	
pH	Oranges	100, 16, 4	0.16	0.25	1.13	0.18	0.15	1.00	1.27	1.40	0.02
	Mandarins	80, 16, 4	0.11	0.74	1.91	0.11	0.74	1.91	1.00	1.50	0.06
	Oranges + mandarins	100, 16, 4	0.15	0.72	1.93	0.17	0.64	1.71	1.28	1.36	
Titratable acidity (% citric acid)	Oranges	100, 15, 4	0.11	0.45	1.36	0.11	0.47	1.36	1.00	1.40	0.004
	Mandarins	100, 15, 4	0.13	0.76	2.08	0.18	0.65	1.50	1.92	1.48*	0.020
	Oranges + mandarins	80, 14, 4	0.14	0.84	2.43	0.18	0.75	1.89	1.65	1.40*	
Maturity index (SSC/TA)	Oranges	140, 16, 4	3.56	0.65	1.69	3.70	0.67	1.63	108	1.36	0.13
	Mandarins	100, 16, 4	1.13	0.79	2.14	1.38	0.68	1.75	1.49	1.48*	0.15
	Oranges + mandarins	160, 16, 4	2.98	0.77	2.08	3.52	0.72	1.76	1.40	1.31*	
BrimA index (%)	Oranges	100, 15, 4	0.85	0.82	2.33	0.89	0.80	2.22	1.10	1.40	0.11
	Mandarins	140, 16, 4	0.70	0.75	2.00	0.79	0.68	1.77	1.27	1.45	0.10
	Oranges + mandarins	140, 14, 4	0.84	0.78	2.15	0.94	0.73	1.93	1.25	1.32	

* Values with significant differences ($P < 0.05$).

Fig. 1. Population distribution of chemical quality parameters for oranges (O), mandarins (M) and oranges and mandarins together (O + M).

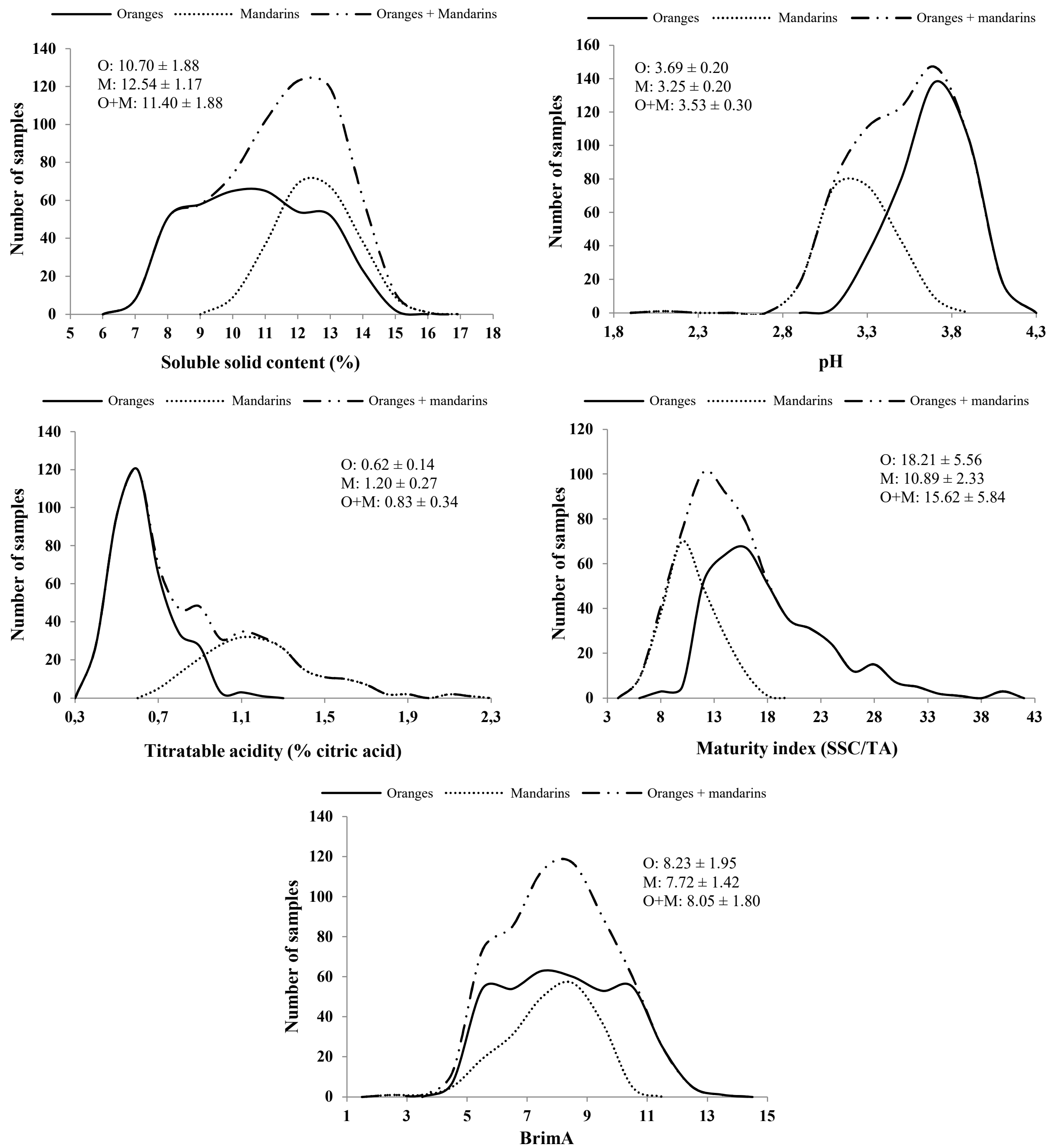

Fig. 2. SEP values obtained for the prediction of chemical quality parameters in the set composed of intact oranges and mandarins using the LOCAL algorithm.

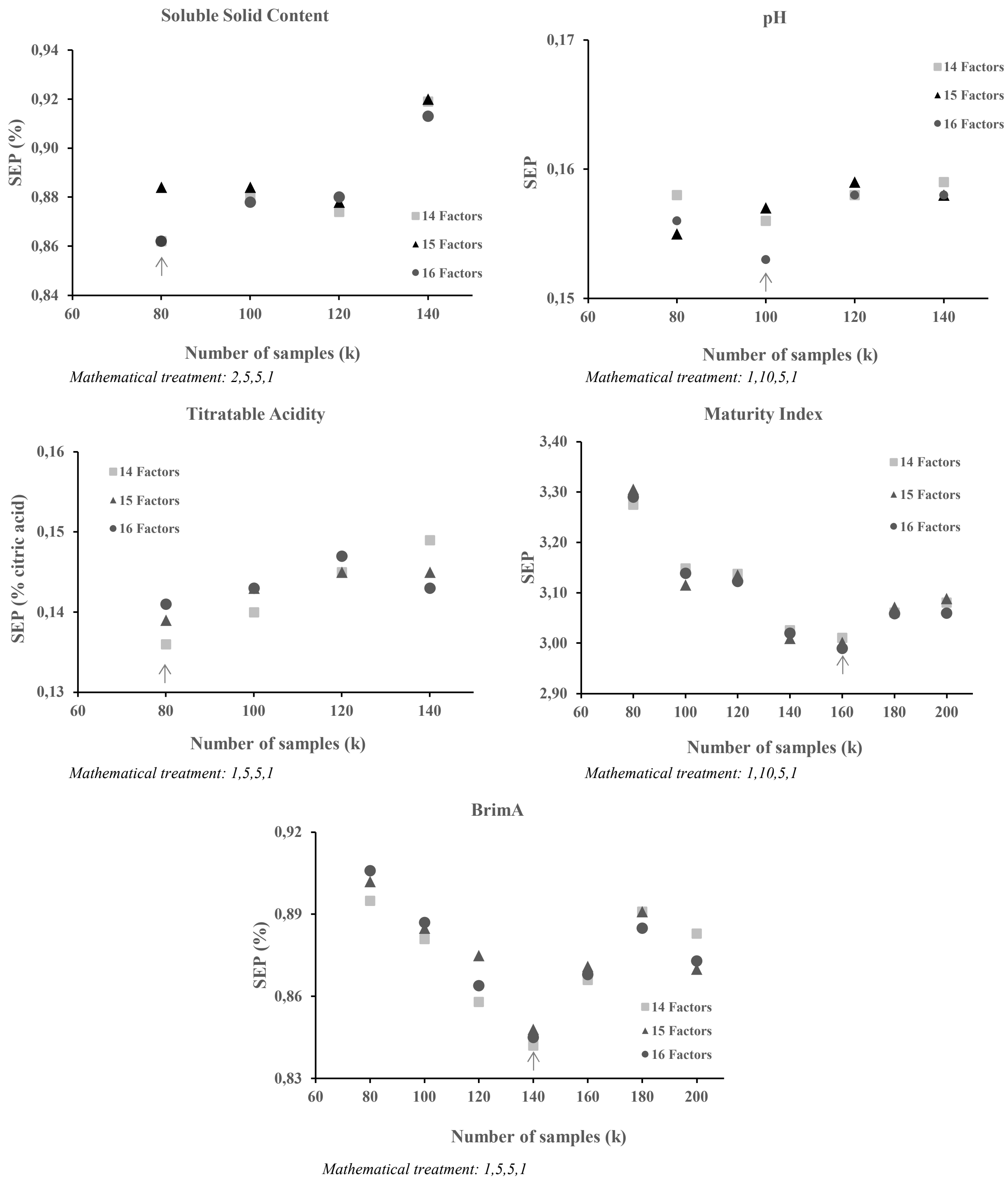

Fig. 3. Loadings for BrimA.